Colonization of Wood by Soil Fungi

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ABSTRACT

Stakes of ponderosa pine (*Pinus ponderosa*) were buried in soil, and each week for 12 weeks isolations were made from them using five agar media. *Fusarium solani, Trichoderma viride, Aspergillus ustus,* and species of *Penicillium* accounted for 92% of all isolates. *F. solani* was the first fungus to invade the wood and was dominant for the first 5 weeks, after which it was replaced by *T. viride* as the dominant species. Soft rot was visible with polarized light after 6 weeks and was well developed after 12 weeks. Small pockets of brown rot were visible to the eye after 10 weeks, and *Trechispora brinkmanni* was isolated after 12 weeks. There were no differences between heartwood and sapwood in susceptibility to invasion by the above fungi and little difference in amount or rate of colonization between the wood at the soil line and the wood at the bottom of the stakes. Russell's medium was selective for *F. solani.*

Wood placed in contact with soil, especially wood that is not of a durable species or that has not been treated with a preservative, usually decays rapidly. This decay is generally attributed to certain fungi in the Basidiomycetes, but usually Ascomycetes and Fungi Imperfecti are involved also. There is a considerable amount of information concerning the fungi associated with decayed posts, poles, railroad ties, and other materials placed in soil. However, most of this information is about the species found in well-decayed wood, and there is little information on the species involved in the initial colonization of the wood or on the rate and frequency with which these species invade the wood. The following study was made to determine the fungi that invaded wood stakes placed in soil, to estimate the rate and frequency with which such fungi invaded the wood, and to find the most suitable media for isolating the fungi.

MATERIALS AND METHODS.—Test stakes 2 × 2 × 25 cm were cut from a kiln-dried board of ponderosa pine (*Pinus ponderosa* Laws.). Each stake was approximately half heartwood and half sapwood. The stakes were numbered, randomized, and driven vertically to a depth of 15 cm in an area about 1.5 m square in an outside soil burial bed at St. Paul, Minn., in June 1963. The burial bed was shaded to maintain favorable soil moisture and to prevent the growth of grass and weeds. The soil in the burial bed contained at least one basidiomycete, *Trechispora brinkmanni* (Bres.) Rogers & Jackson, as determined by isolations and fruit body formation around test stakes in previous studies.

Each week after burial except the tenth, five stakes were removed, scrubbed thoroughly under running water, wiped dry with paper towels, dipped in 95% ethyl alcohol, and flamed. At the soil line and at a point 2.5 cm from the bottom of each stake, cuts were made with a sterile scalpel into opposite sides of the stake into the heartwood and sapwood to a depth of about 3 mm. From the centers of each of these freshly exposed surfaces, two 1-2-mm cubes of wood were cut and placed on each of the five following media: (i) acidified weak malt agar (0.1 g malt extract, 2 g agar, 0.2 ml 50% lactic acid, 100 ml distilled water); (ii) malt-salt agar (1 g malt extract, 2 g agar, 7 g NaCl, 100 ml distilled water); (iii) sawdust agar (1 g sawdust from *Pinus resinosa,* 2 g agar, 100 ml distilled water); (iv) Russell's medium (12); and (v) PCNB agar (5).

The plates were incubated at room temperature and examined microscopically after 4 days and every second day thereafter for 3 weeks. Fungi that did not sporulate or that were not readily identifiable were transferred to fresh plates of malt agar until identified.

RESULTS.—The results of the isolations, per week on each medium, are summarized in Table 1. The values are the total number of times each species was isolated from the wood at the soil line and at the bottoms of the stakes and from sapwood and heartwood of five stakes. No fungi were isolated from control stakes treated in the same manner but not buried.

The most prevalent fungi invading the wood were *Fusarium solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. and *Trichoderma viride* Pers. during the first week, *Alternaria humicola* Oud., *Aspergillus niger* v. Tiegh., and species of *Penicillium* during the second week, and *Aspergillus ustus* (Bainier) Thom & Church during the third week. *F. solani* was isolated most frequently; it was the first fungus to invade the wood and was the predominant species for the first 5 weeks, after which *T. viride* became predominant. *F. solani, T. viride, Aspergillus ustus,* and *Penicillium* spp. accounted for 92% of all isolates; most other species were encountered infrequently.

All fungi for which there were adequate data to judge were isolated with equal frequency from the heartwood and the sapwood. There was little difference in fungi isolated from the wood at the soil line and the wood at the bottoms of the stakes, except that *Alternaria humicola, Aspergillus niger,* and *Penicillium* spp. were isolated somewhat more frequently from the wood at the soil line than from the bottoms of the stakes.

There were differences in numbers of species isolated depending on the medium used. Acidified weak malt agar and sawdust agar yielded the greatest numbers. No one medium gave a complete picture of the fungus population of the stakes. Russell's medium was selective for *F. solani,* which formed constricted, nonsporulating colonies after about 2 weeks.

TABLE 1. Frequency of isolation of various fungi from ponderosa pine stakes, per week and per medium. The values given are totals for five stakes and include all isolations from both the wood at the soil line and the wood at the bottoms of the stakes and from both heartwood and sapwood

	Total number of times each species was isolated											
	June	July				August				September		
Medium and species	30	7	14	21	28	4	11	18	25	2	9	16
Acidified weak malt agar												
Fusarium solani	20	24	34	36	11	15	6	3		10	15	16
Trichoderma viride	7	23	19	18	26	26	26	34		31	31	27
Aspergillus niger	0	7	1	0	0	0	0	0		0	3	0
A. ustus	0	0	10	21	6	8	13	10		12	7	5
A. wentii Wehm.	0	1	0	0	0	0	0	0		0	0	0
Penicillium spp.	0	13	2	5	6	2	15	9		6	6	6
Alternaria humicola	0	2	4	2	1	3	0	0		2	1	1
Absidia sp.	1	0	0	0	3	12	1	1		2	0	3
Rhizopus sp.	0	2	0	0	0	0	0	0		0	0	0
Verticillium sp.	0	1	0	0	0	0	0	0		0	0	0
Rhizoctonia sp.	0	0	0	0	0	0	8	0		0	0	0
Trechispora brinkmanni	0	0	0	0	0	0	0	0		0	0	3
Malt-salt agar												
Fusarium solani	22	28	15	14	15	4	7	13		2	7	11
Trichoderma viride	5	22	0	1	7	0	2	3		1	0	2
Aspergillus niger	0	6	3	0	4	4	2	16		5	5	3
A. ustus	0	0	28	28	23	10	17	11		30	28	29
Penicillium spp.	0	9	10	4	2	7	9	6		6	13	7
Alternaria humicola	6	7	5	11	3	0	0	2		2	3	4
Aspergillus ruber (Spieck & Brem.) Thom & Church	0	0	1	0	0	0	0	0		0	0	0
Absidia sp.	0	0	1	0	0	0	0	0		0	0	0
Mucor sp.	3	4	0	0	6	14	4	6		5	0	4
Rhinotrichum sp.	0	2	0	0	0	0	0	0		0	0	0
Spicaria divaricata (Thom) Gilman & Abbott	0	0	0	0	0	0	0	0		1	0	0
Sawdust agar												
Fusarium solani	26	30	37	32	20	31	18	11		12	18	27
Trichoderma viride	3	17	9	9	18	15	28	34		26	24	19
Aspergillus niger	0	5	0	0	0	0	0	0		0	0	0
A. ustus	0	0	11	10	9	2	18	4		6	8	3
Penicillium spp.	1	5	2	3	4	2	4	1		5	9	3
Alternaria humicola	0	3	0	0	0	0	0	1		0	1	0
Cephalosporium curtipes Sacc.	0	0	0	1	0	0	0	0		0	0	0
Pullularia sp.	0	0	0	0	0	0	2	0		0	0	0
Trechispora brinkmanni	0	0	0	0	0	0	0	0		0	0	2
Absidia sp.	0	0	0	0	0	5	0	0		0	0	0
PCNB agar												
Fusarium solani	22	31	32	35	37	35	32	30		36	36	38
Trichoderma viride	6	8	19	14	25	22	24	28		27	26	24
Aspergillus niger	0	0	0	0	0	0	0	0		2	0	0
A. ustus	0	0	1	1	1	2	0	0		0	0	0
Penicillium spp.	0	5	0	1	0	0	4	0		4	2	2
Alternaria humicola	0	0	2	0	0	0	0	0		0	0	0
Dactylosporium sp.	0	0	0	0	0	0	0	1		0	0	0
Russell's medium												
Fusarium solani	14	22	24	29	12	9	5	1		6	9	18

Soft rot was visible with polarized light (2, 3, 13) after 6 weeks, was well developed after 12 weeks, and was confined mainly to the summer wood. The fungus or fungi causing the soft rot were not determined. Small pockets of brown rot, about 2 mm in diam, were visible after 10 weeks, and *Trechispora brinkmanni*, a brown-rotter, was isolated after 12 weeks. Although the stakes were discolored with blue stain, no blue-staining fungi, except possibly *Alternaria humicola* and *Pullularia* sp., were isolated. The species of *Penicillium* isolated caused a distinct pink stain.

DISCUSSION.—If different species of wood or different media had been used, undoubtedly the results of the isolations would have been different, but several points are worth noting.

The total number of species recovered from the wood was small in comparison to the number that can be recovered directly from the soil by dilution plate techniques (unpublished data). The number of species of importance in colonizing wood seemed to be even fewer, since the majority of the species isolated accounted for only a small portion of the total. That species of *Mucor*,

Absidia, and *Rhizopus* were isolated from the wood was surprising, since these species are not usually considered to be wood inhabitants. *Chaetomium globosum* Kunze was not isolated, even though this fungus had always been recovered from wood and wood fiber products buried in the same soil. This may be due to the inability of the species to colonize ponderosa pine.

The population of *F. solani* in the wood decreased from the fourth to the eighth week, after which it increased again. This decrease could not be attributed to temperature or moisture effects and was probably due to the increase and decline of populations of antagonistic organisms, possibly either bacteria or actinomycetes, since a few isolations, not reported here, indicated that populations of such organisms were present in the wood.

Mangenot and Reymond (8) concluded that species of *Trechispora* were the most important organisms degrading lignified materials in soil in France. *T. brinkmanni* was the basidiomycete most frequently isolated from the soil of Wicken Fen, England (14). Species of *Trechispora*, including *T. brinkmanni*, were also found in Australian soils (15). *T. brinkmanni* is the only basidiomycete isolated from the outside soil burial bed at St. Paul, and it has formed prominent fruit bodies around test stakes in nearly every study since 1958. Apparently this species and other species in this genus occur in soils in many places of the world.

Species of *Aspergillus, Fusarium, Penicillium*, and *Trichoderma* are among the first invaders of wood in soil and are often able to inhibit basidiomycetes (8, 11). Although several species of the Ascomycetes and Fungi Imperfecti have been shown to decay wood (3, 4, 9, 10), in nature they are eventually replaced by the basidiomycetes. The reasons for this succession have not been determined. The role of Ascomycetes and Fungi Imperfecti in wood deterioration, especially in relation to succession of fungi in wood, should be studied, particularly in view of recent reports that these fungi can detoxify wood preservatives and the naturally occurring heartwood toxins (1, 6, 7) and thus pave the way for the eventual decay of the wood by the basidiomycetes.

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